Standard PCR (From BV's Cookbook)

Setting up the reactions:

Make a master mix by setting up the following for each 10ul reaction:

H_2O	q.s. to 10 ul
10xPCR buffer	1 ul
dNTP(10 mM each; from BRL)	1 ul
DMSO	0.6 ul
Primer 1(50 uM)	0.2 ul
Primer 2 (50 uM)	0.2 ul

Add each reagent in the order listed and mix the reaction vigorously.

Add Platinum Taq, 5 u/ul (BRL)	0.05 - 0.1 ul

Add the above mix to 1 ul template DNA, 5 - 20 ng/ul
Always include a H2O negative and a positive control for your PCR
Add 1 drops light mineral oil to each tube with a plastic transfer pipette.
Place tubes in PCR machine.

Running the reactions:

The following parameters are a good starting point

Denaturation: one cycle of 94°, 1minute

Polymerization: 35 cycles of:

Denaturing	94°C	1 min
Annealing	55°C	15 sec
Extension	70°C	15 sec

(Add 1 minute to extension for each kb of PCR product).

Extension:

One cycle of 70°C, 5min.

Notes:

1. Make sure your primers are designed as outlined the "Designing Primers for PCR protocol", and that they have a T_m of ~55-60°C.

2. You may want to try lower annealing temperatures which will vary with the primers used. You can vary by 4°C intervals until the best temperature is achieved. Time of annealing does not make much difference after 1min. Shorter times may not provide a reproducible plateau while longer time does not improve outcome.

3. Lower number of cycles may provide fewer products and less background. Yield drops off after 30 cycles and Taq may already be degraded by then.

4. Hai tested the effect of TE (10 mM Tris, 1 mM EDTA, pH 7.4, from Quality Biologics, Cat nol 351: 010-130), and found that 1, 2, 3, or even 5 ul of this TE, in a 25 ul total PCR reaction, did not inhibit the efficiency of a 1.5 kb amplification (5 ul TE may even have helped!).

5. With high GC templates, you can try higher concentrations of DMSO (8%, 10%, 12%). This sometimes works.

10X PCR Buffer (DMSO protocol)

NH_4SO_4	166mM
Tris, pH 8.8	670mM
$MgCl_2$	67mM
2-mercaptoethanol.	100mM